1999). Abstracts for these references evidencing their publication dates are submitted herewith in Appendix B.

In the Specification

Please amend the specification as follows:

1. Please re-write the two paragraphs starting on page 10, line 24, as follows:

Figure 7 is a graph depicting the effect of different adjuvants on total IgG titers of anti-HBs, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT), *Escherichia coli* heat-labile enterotoxin (LT), the B subunit of Cholera toxin (CTB), a detoxified mutant of *Escherichia coli* heat-labile enterotoxin (LTK63), CpG oligonucleotide (motif #1826, SEQ ID NO. 90) or non-CpG control oligonucleotide (motif #1982, SEQ ID NO. 91) as adjuvants (1, 10 or 500 μg). In groups which responded, all mice gave titers > 10, except in the case of 10 μg LT where only 1/5 mice responded.

Figure 8 is a bar graph depicting the effect of different prime/boost strategies on total IgG titers of anti-HBs, wherein BALB/c mice were immunized: (i) by IM injection with HBsAg (1 μg) in combination with alum plus CpG oligonucleotide (motif #1826, SEQ ID NO. 90) and boosted at 4 weeks as prime, or by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT) and /or CpG oligonucleotide (motif #1826, SEQ ID NO. 90); or (ii) by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT) and /or CpG oligonucleotide (motif #1826, SEQ ID NO. 90) and boosted at 4 weeks as prime or by IM injection with HBsAg (1 μg) in combination with alum plus CpG oligonucleotide (motif #1826, SEQ ID NO. 90). Numbers at the top of each bar represent the IgGa/IgG1 ratio.

2. Please re-write the paragraph starting on page 30, line 12, as follows:

Other medically relevant microorganisms have been described extensively in the literature, e.g., see C.G.A Thomas, *Medical Microbiology*, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference.

3. Please re-write the paragraph starting on page 40, line 24, as follows:

The invention also utilizes polynucleotides encoding the antigenic polypeptides. It is envisioned that the antigen may be delivered to the subject in a nucleic acid molecule which encodes for the antigen such that the antigen must be expressed *in vivo*. Such antigens delivered to the subject in a nucleic acid vector are referred to as "antigens encoded by a nucleic acid vector." The nucleic acid encoding the antigen is operatively linked to a gene expression sequence which directs the expression of the antigen

nucleic acid within a eukaryotic cell. The "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the antigen nucleic acid to which it is operatively linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPTR), adenosine deaminase, pyruvate kinase, β-actin promoter and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the cytomegalovirus (CMV), simian virus (e.g., SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

4. Please re-write the paragraph starting on page 50, line 28, as follows:

Subject doses of the compounds described herein typically range from about 80 μg/day to 16,000 μg/day, more typically from about 800 μg/day to 8000 μg/day, and most typically from about 800 μg/day to 4000 μg/day. Stated in terms of subject body weight, typical dosages range from about 1 to 200 μg/kg/day, more typically from about 10 to 100 μg/kg/day, and most typically from about 10 to 50 μg/kg/day. Stated in terms of subject body surface areas, typical dosages range from about 40 to 8000 $\mu g/m^2$/day, more typically from about 400 to 4000 $\mu g/m^2$/day, and most typically from about 400 to 2000 $\mu g/m^2$/day.

5. Please re-write the paragraphs starting on page 57, line 16, and ending on page 59, line 31, as follows:

2. Mucosal Immunization

Each animal was immunized with 1 or 10 ug plasma-derived HBV S protein (HBsAg, ad subtype, Genzyme Diagnostics, San Carlos, CA), which was administered alone or in combination with 1 or 10 μg of CT or LT or derivative of them and/or CpG oligonucleotide #1826. The derivatives of CT were the B subunit of CT (CTB). The detoxified derivatives of LT were all produced by genetic mutations that affected the A subunit or enzymatic activity and included LTK63. All vaccines were delivered in a total

volume of 150 µl, which was applied as droplets directly over both external nares of lightly anaesthetized mice. Some mice were boosted in the identical manner at 8 weeks after prime. All experimental groups contained 5 or 10 mice.

3. Collection of samples

Plasma: Plasma was recovered from mice at various times after immunization (1, 2, 4 and 8 wk post-prime and 1, 2 and 4 wk post-boost) by retro-orbital bleeding and stored at -20°C until assayed.

Fecal pellets: Fecal pellets were collected from mice at various times after immunization (1, 2, 4 and 8 wk post-prime and 1, 2 and 4 wk post-boost). Mice were isolated in individual cages without bedding for a 24 hr period, following which fecal pellets were collected and weighed into 0.1 mg aliquots. One ml TBS (0.05 M Tris-HCl, 0.15 M NaCl, pH 7.5) and 0.1 µg sodium azide (Sigma) were added per 0.1 mg of fecal material. Samples were allowed to rehydrate for 30 min at RT, then were centrifuged at 6000 rpm for 15 min. to remove fecal debris and supernatants were collected and stored at –20°C until assayed for S-IgA by ELISA.

Lung washes: Lung washes were carried out on mice 4 wk after primary immunization or boost. A 0.33 cc Insulin syringe with a 29G1/2 needle attached (Becton Dickinson, Franklin Lakes, NJ) was used for carrying out lung washes. One ml TBS was drawn into the syringe and a length of polyethylene (PE) tubing that was 1 cm longer than the needle was attached (PE20, ID = 0.38 mm, Becton Dickinson). The mouse was killed by anesthetic overdose and the trachea was immediately exposed through an anterior midline incision made using fine-tipped surgical scissors (Fine Science Tools Inc., North Vancouver, BC). A small incision was then made in the trachea and a clamp (Fine Science Tools Inc., North Vancouver, BC) was placed above it. The PE tubing was passed a few mm down the trachea through the incision and a second clamp was placed just below the incision to hold the PE tubing in place in the trachea. The TBS solution was slowly instilled in the lungs then withdrawn three times (80% recovery expected). Recovered samples were centrifuge at 13,000 rpm for 7 min., and the supernatants were collected and stored at –20°C until assayed by ELISA.

4. Evaluation of immune responses

Systemic humoral response: HBsAg-specific antibodies (anti-HBs) in the mouse plasma were detected and quantified by end-point dilution ELISA assay (in triplicate) for individual animals as described previously (Davis *et al.*, 1998). Briefly, 96-well polystyrene plates (Corning) coated overnight (RT) with plasma derived HBsAg particles (as used for immunization) (100 µl of 1 µg/ml in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6) were incubated with the plasma for 1 hr at 37°C. Captured antibodies were then detected with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, IgG1 or IgG2a (1:4000 in PBS-Tween, 10% PBS: 100 µl/well; Southern Biotechnology Inc., Birmingham,

AL), followed by addition of o-phenylenediamine dihydrochloride solution (OPD, Sigma), 100 µl/well, for 30 min at RT in the dark. The reaction was stopped by the addition of 4 N H_2SO_4, 50 µl/well.

End-point dilution titers were defined as the highest plasma dilution that resulted in an absorbance value (OD 450) two times greater than that of non-immune plasma, with a cut-off value of 0.05. Anti-HBs titers of responding mice (endpoint titers > 10) were expressed as means SEM of individual animal values, which were themselves the average of triplicate assays.

Mucosal humoral response: This was carried out on fecal supernatants or recovered lung washes as for plasma (above) except samples were incubated on coated plates for 2 hr at 37°C and captured antibodies were detected with HRP-conjugated goat anti-mouse IgA (1:1000 in PBS-Tween. 10% PBS: 100 µl/well; Southern Biotechnology Inc). Non-immune fecal pellet or lung wash solutions were used to determine negative control values. For lung wash solutions, anti-HBs endpoint dilution titers were reported (as described above), whereas for fecal pellet solutions, absorbance values (OD 450) greater than that of non-immune fecal pellet solution were calculated and expressed as mean SEM of individual OD 450 values, which were themselves the average of triplicate assays.

Evaluation of CTL responses: Spleens were removed from mice 4 wk after primary immunization or boost. *In vitro* assay of HBsAg-specific cytolytic activity was carried out as previously described (Davis *et al.*, 1998). In brief, single cell suspensions were prepared and suspended in tissue culture medium (RPMI 1640, 10 % FBS, Life Technologies, Grand Island, NY, supplemented with penicillin-streptomycin solution, 1000 U/ml, 1mg/ml final concentrations respectively, Sigma). Splenocytes (3 x 10^7) were co-cultured for 5 days (37°C, 5% CO2) with 1.5 x 10^6 syngeneic HBsAg-expressing stimulator cells (P815-preS, generously provided by F. V. Chisari, Scripps Institute, La Jolla, CA) that had been previously inactivated by irradiation (20 000 rad). Effector cells were harvested, washed, serially diluted and cultured with 5 x 10^4 ^{51}Cr-labeled HBsAg-expressing target cells (P815S) in round bottom 96-well culture plates (37°C, 5% CO2, 4 hr). Supernatant (100 µl) was removed for radiation (gamma) counting. Spontaneous release was determined by incubating target cells without effector cells and total release by addition of 100 µl 2 N HCl to the target cells. The percent lysis was calculated as [(experimental release - spontaneous release)/(total release - spontaneous release)] x 100. The percent specific lysis was calculated as % lysis with P815S - % lysis with P815 cells. CTL activity for responding mice [% specific lysis > 10 at effector:target (E:T) of 25:1] were expressed as mean SEM of individual animal values, which were themselves the average of triplicate assays.

In the Claims

Please re-write the pending claims as follows: